1: INTRODUCTION:

Cancer is determined by uncontrolled growth of cells (Shubharani *et al*., 2014) and is possibly connected due to the participation of oxidant species created by normal endogenous metabolic byproducts as well as harmful exogenous agents which modifies the biomolecules (Devasagayam *et al*., 2005; Rizzo *et al*., 2010). The incidence of cancer has been estimated to rise in many third world countries by 100% to 180% in the next 15 years (Khatib and Aljurf, 2008)and 15 million new cases are ascending with the expected death of 10 million by the year 2020 (Kamangar *et al.,* 2006) due to the exposure of human beings to many harmful chemicals. About 10% of the total chemicals which are in use today (approximate number of these chemicals is 85,000) are toxic and imparts cytotoxicity to living organisms and predicted different malformations/diseases (Pimental *et al.,* 2007). Amongst all the diseases, cancer is the most challenging degenerative disease leading to morbidity and mortality in the entire world today. It is also claimed to have taken the lives of about 200 million worldwide by 2015 (Rajesh *et al*., 2011), the reason behind it is because of the certification of abnormal cells raised from the increased proliferation, insufficient apoptosis or combination of both (Abdul *et al*., 2009).Despite a lot of research has been made by researchers of several disciplines for the past several years for the prevention of cancer, no effective therapy has been discovered till now, WHO, 2008; World Cancer Research Fund, 2007.

There are over 100 different kinds of cancer known that affects human beings (National Cancer Institute, 2014). The main types of cancer in males are lung cancer, prostate cancer, colorectal cancer and stomach cancer whereas in females breast cancer, colorectal cancer, lung cancer and cervical cancer are the most common types of cancer. The rates of cancer are increasing frighteningly as more people live to an old age as well as changing lifestyles in the developing countries (Jemal *et*

al., 2011). 90-95% of the cancer is due to environmental factors such as use of tobacco (25-30%), obesity(30-35%), infections(15-20%), radiation(both ionizing and non ionizing radiations upto 10%), stress, lack of physical activity, environmental pollutants and remaining 5-10% are due to inherited genetics (Anand *et al.,* 2008). There is multiple possible reason of cancer and exposure to particular substances has been linked to cause different types of cancer and such substances are called as carcinogens. Cancers arises from the assembly of several mutations causing genetic alterations thereby caused failure of the regulation of cell growth and ultimately leads to clonal expansion from its primary sides to other organs, a term called as metastasis and most of the metastasis occurs in lung, liver, brain and bones (National Comprehensive Cancer Network, 2013).

Various kinds of active measures have been taken to decrease the risk of cancer (Cancer Prevention: 7 steps to reduce your risk, 2010) and about 30% of the cancers have been estimated to prevent by avoiding the risk factors causing cancer (World Health Organisation, 2011). Many treatment options for cancer and one amongst them is chemotherapy. Chemotherapy is the treatment of cancer with one or more cytotoxic anti-neoplastic drugs and such treatments lengthened the life or may permanently cure the patients however it is often limited by toxicity to other tissues in the body causing miserable pain, blood clots fatigue and infection (McMillen and Matt 2013) eventually leading to death because of failing organs and immunosuppression (Ugbogu *et al*., 2013). The use of these drugs also generates free radicals (ROS and RNS) (De Flora *et al*., 1992, Conklin, 2004) and the production of ROS/ RNS beyond neutralizing limits is augmented due to changing lifestyles, the ultimate result is the related fall in the inherent anti-oxidative defense system that conveys oxidative stress (Fang *et al*., 2002, Prochaska *et al*., 1992, Surh *et al*., 2005, Singh *et al*., 2015). FDA, US has approved about 132 cancerschemotherapeutic drugs in whichoxidative stress have been

reported to cause by 56 drugs(Chen *et al*., 2007). The major limitation of cancer chemotherapy is that it not only targets the cancer cells, but also target normal cells and such side effects leads to various complications during and after treatment (Agur *et al*., 1988., Markin, 2014., Livshits *et al*., 2014). The increasing frequency of oxidative stress has become an obligatory field to prevent it with the use of exogenous antioxidants to reduce it (Zhao *et al*., 2010; Jermal, *et al*., 2009, Conklin, 2004). Mammalian cells are gifted with various endogenous antioxidants such as enzymatic (superoxide dismutase, catalase, glutathione peroxidase and glutathione reductase) as well as non enzymatic [glutathione, vitamin E (tocotrienols and tocopherols), vitamin C] antioxidants (Valluru *et al*., 2014; Yu *et al.,* 1992) that neutralize the free radicals (Ahmad *et al.,* 2010). They counterbalance the ROS/RNS mediated injuries, but when these free radicals outnumbered the defense systems, it leads to oxidative stress (Tarlovsky, 2012; Dalermelina *et al*., 2014). The free radicals act as oxidants which are cytotoxic at pathogenic level, but when present in controlled conditions they serve as signalling and regulatory molecules at physiological levels (Pham-Huy *et al*., 2008).Free radicals cause normal tissue damage by reacting with lipids in cellular membranes, nucleotides in DNA, sulphahydril groups in proteins, crosslink ribonucleoproteins and carbohydrates (Dizdaroglu *et al*., 2002; Hollstein *et al*., 1991; Cooke *et al*., 2003; Grigorov, 2012; Shobha *et al*., 2013). Such changes in the morphological and metabolic process of normal cells ultimately lead to mutation, transformation and ultimately lead to cancer (Devasagayam *et al.,* 2004, Lobo *et al*., 2010). Free radicals attacks double bonds of pyrimidines and remove hydrogen from sugar moities resulting in chain reaction of DNA, ultimately leading to mutagenesis and carcinogenesis (Lobo *et al*.,2010). Mutations are not only the trademark of cancer but are also the center of how cancer evolves (Keith *et al*., 1999). A process by which simultaneous changes occur in the DNA of an organism is called mutagenesis and agents

causing mutation are called mutagens or clastogens (Sharma and Aggarwal, 2015; Singh *et al*., 2015; Machlin and Bendich, 1987). Clastogens caused structural chromosomal damage and if it is not repaired it may be the foundation of mutation whose cellular expression leads to many harmful diseases (Singh *et al*., 2015; Teaf and Middendroff, 2000).

Cyclophosphamide is a well known nitrogen mustard alkylating agent (Takimoto *et al,* 2005) used as a chemotherapeutic agent (Baumann and Preiss2001; Fleming 1997) and used in the treatment of various types of cancers like leukemia (Rao *et al.,* 2005), breast cancer (Pritchard *et al.,* 1997), lung cancer (Chrystal *et al.,* 2004), lymphomas (Escalon *et al.,* 2005), prostate cancer (Nicolini *et al.,* 2004), ovarian cancer (Stiff *et al.,* 2004) and multiple myeloma (Dimopoulos *et al.,* 2004). Besides its beneficial effects cyclophosphamide causes adverse side effects like nausea and vomiting (Singh *et al*.,1991), bone marrow suppression (Lohrmann, 1984), toxicity to kidney tubules, urine channels, heart and liver (Morandi *et al*., 2005;Papaldo *et al.*, 2005; Schwartz *et al*.,2005; Amudha *et al*.,2007). Moreover, International Agency for Research Centre (IARC) has acknowledged CP act as a carcinogen for both animals and humans (IARC, 1987). Cyclophosphamide metabolism in the liver are done by mitochondrial enzymes cytochrome P450 breaks down cyclophosphamide into three metabolites such as; 4 hydroxycylophosphamide (has chemotherapeutic activity: Huttunen *et al.*, 2011), acrolein and phosphoramide (Reactive oxygen species: Hales, 1982; Sladek, 1971, 1988). The intermediates act by modifying and cause tissue injuries (Abraham and Sugumar, 2008), cross link the adenine and guanine bases in DNA thus inhibiting DNA, RNA and protein synthesis and decreases the antioxidants capacity of cells (Ray *et al*., 2010). Several studies suggested that the supplementation of external antioxidants obtained from plant can restore

the responses given by the treatment of such antineoplastic drugs (Wijl *et al.*, 1997).

Many researchers have investigated that the increase levels of antioxidants present in plants are believed to decrease the oxidative damage and its harmful effects (Bjelakovic *et al.,* 2007). There is increasing consciousness and attention in the antioxidant activities and potential health benefits of plants because they are the outstanding reservoir of secondary metabolites linked in the prevention of many diseases including cancer (Nirmala *et al*., 2011; Fulda and Efferth , 2015) diabetes, cardiovascular problems(Robbins, 2003). Plant secondary metabolites that minimize the detrimental effect of mutagens are called as antimutagens/ anticarcinogens (Mitcher *et al*., 1986; Kaur *et al*., 2011; Nirmala, *et al*., 2011 ;) and that neutralizes free radicals are termed as antioxidants (Kaur *et al*., 2011).

So there is growing alarm in the search for and investigation of natural substances of plant origin that posses antimutagenic, anti-genotoxic and anti-oxidant activities against inducers of reactive oxygen species. Biological and pharmacological activities are the biochemical properties of medicinal plants that protect the cells against oxidative induced damage (Mierlici *et al*., 2009; Agrabeyli, 2012; Al-Awaida and Akash, 2015). Synthetic antioxidants such as butylated hydroxytoluene (BHT), butylated hydroxyanisole (BHA) are preferable but can cause serious ill effects in human health as per recent reports given by Lobo *et al.,* 2010; Aksoy *et al*., 2013.

So, it has been suggested to use the plants of medicinal properties to fightagainst the free radicals/mutagens by inducing phase II enzymes that can reduce the action of initiation, promotion or progression stages of cancer and other degenerative diseases (Hong and Sporn, 1997; Tan and Spivack, 2009; Kundu and Surh, 2009;Edenharder *et al*., 1993)**.** Phase II enzymes help in the elimination of the elctrophillic chemicals produced by mutagens/ carcinogens, catalyzing the conjugation by sulfation,

glucuronidation or glutathionylation (Kwak *et al*., 2004). Medicinal plantsare less toxic and havefewer side effects (Johnson, 2007) and they can be easily metabolised inside the body without any harmful effects that leads to the phytochemical based remedies (Sangwan *et al*., 1998; Ma and Kineer, 2002; De Flora and Ferguson, 2005; Anetor *et al*., 2008). Also the plants are rich source of secondary metabolites such as flavonoids, phenolics, carotenoids, coumarins, anthraquinones, tannins, terpenoids, saponins that play a prominent role in inhibiting human carcinogenesis and repair the cell mutations(Ruan, 1989) and they have been using as a first line of cancer fighting agent in developing countries (Sawadogo *et al*., 2012).

Plants based chemoprevention ameliorate the effect of mutagens/ carcinogens into two main mechanisms; first: it prevents the onset of cancer by detoxifying, modifying the carcinogens uptake and metabolism, scavenging the reactive oxygen species and enhancing the DNA repair process; second, it suppresses the inhibition, promotion and progression of mutagenesis/ carcinogenesis leading to cancer (Greenwald, 2004; Surh, 2003; Wattenberg, 1985). Usually adverse side effects of such antineoplastic drugs can be prevented by the intervention of external antioxidants obtained from plants (Gentile *et al*., 1998; Wijl *et al.*, 1997). In general all antioxidants have been suggested to inhibit the process of mutagenesis and carcinogenesis (Ferguson, 1994). Many antimutagenic plants have also been shown to help in the prevention of cancer and other diseases (Berhow *et al*., 2000; Nishino, 1998, Patel *et al*., 2007, Surh and Ferguson, 2003).Various genetic biomarkers and test-systems have been employed to estimate the genotoxic effects and antigenotoxic potential of medicinal herbs and phytochemicals in order to standardize their consumption (Gateva *et al*., 2014, Angelova *et al.,* 2014, Todorova *et al*., 2014. Valuable and representative information about the biological activity and anti-genotoxic potential of medical plant extracts could be provided by combined studies with in vitro and in vivo test-systems.

Enormous amount of plants has not been evaluated for their antimutagenic and antioxidant activities still, although many of the plant species have been suggested to have rich amount of phytochemicals. So we aimed to investigate the antimutagenic and antioxidant potential of the rizhomes of *Curcuma caesia* Roxb. in the present study.

Curcuma caesia Roxb. also known as black turmeric is a perennial herb with bluish black rhizomes and it is famous for its medicinal properties. It is recognized as a medicinal herb to possess with various properties such as anti-fungal activity by Banerjee and Nigam, 1976,smooth muscle relaxant and anti-asthmatic activitybyArulmozhi *et al.,* 2006, bronchodilating activity by Paliwal *et al.*, 2011, antioxidant activity by Mangla *et al.*, 2010, anxiolytic and CNS depressant activity, locomotor depressant, anti-convulsant by Karmakar *et al.*, 2011,anthelmintic activity by Gill *et al.*, 2011,anti-bacterial activityby Rajamma *et al*., 2012,anti-ulcer activityby Das *et al*., 2012. The phytochemical studies of *Curcuma caesia* revealed the presence of multiple phytoconstituents like essential oils with camphor, ar-turmerone,(Z) ocemene, ar-curcumene,1,8-cineole, elemene, borneol, bornyl acetate, curcumene etc (Panday and Chowdhary, 2003).

Scanty information is available regarding the bioactivities *of C. caesia* Roxb, till now there is no information on the antimutagenic and antigenotoxic activities of *C. caesia* Roxb. Keeping this in view as well our interest in antimutagens and antioxidants and potential ability of plant secondary metabolites to serve as probable antimutagens and antioxidants, the present study was planned to meet the following objectives:

1. Preparation of different solvent extracts from rhizomes and preliminary phytochemical screening.
2. Evaluation of Antioxidant and Antimutagenic activity of plant extracts.
3. Evaluation of Antigenotoxic activities of the plant extracts.
4. Isolation and characterization of bioactive constituents in most active fractions using chromatographic and spectroscopic techniques